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Juglone's effect on germination and cellular respiration rates of soybean and corn varieties

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Abstract

Ohio produces ~250,000 tons of black walnuts yearly with no major commercial use. Black walnuts contain a toxic compound called *juglone*, which hinders the growth of many but not all plants. This project examines the effects of black walnut extracts on *Glycine max* (soybeans) and *Zea mays* (corn) varieties grown in Ohio. The hypothesis is that the extracts derived from the black walnut products will enhance the germination, cellular respiration, and growth of *glycine* and *zea*. Measured amounts of hull and nut extracts derived from whole black walnuts were added to the seeds of *glycine* and three *zea* (Ambrosia, True Gold, Painted Hill) varieties to examine their effects on germination. Cellular respiration of grouped seedlings was measured using Vernier CO_2 probes to estimate the amount of oxygen utilized and energy produced during germination. Furthermore, the shoot and root lengths of extract-treated plants were measured at different time intervals to examine the plant growth. Data were analyzed and plotted in Microsoft Excel comparing water-treated and extract-treated conditions. The results demonstrate that the hull extract, and not the nut extract, derived from black walnuts predominantly increased the germination, cellular respiration rate, and overall growth of *glycine* and *zea* varieties. These findings shed new light into the effect of juglone on *glycine* and *zea* health and demonstrate the potential for use of black walnut products as biofertilizers in Ohio *glycine* and *zea* fields.

Acknowledgements

I like to thank my Science Honors Teacher Mrs. Wendy Pinta for her motivation, guidance, and mentoring she offered during this project. I also thank her for demonstrating the use of *Vernier* CO_2 probes and *Vernier Graphical Analysis 4* App. I like to thank Dr. Raj Govindarajan at the Ohio State University for research supplies used in the project and for data analysis with Microsoft Excel. I thank both Mrs. Pinta and Dr. Govindarajan for editing and proofreading this document.

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Introduction

Each year, hundreds of black walnuts crash down in my backyard. Every time, I had to manually pick every one of them up, and this got me thinking, “How did one tree produce so many black walnuts?” The tree provided lots of shade in my backyard, and interestingly, no other plants grew beneath it. I didn’t know if this was caused by the lack of sunlight or something in the black walnut tree that prevented the growth of other plants. The squirrels would always peel the hull and eat the nut inside, and the hulls would stay on the ground making stains and messy appearances, further killing the plants around them. This repeats every fall, and this year I decided to do something about this constant hassle. Reading about black walnut trees, I came across the fact that there are millions of black walnut trees in Ohio, and these trees may produce some harmful substances to stun the growth of nearby plants, thereby facilitating its own growth. This was fascinating to me, and I decided to do in-depth research on this interaction for my science fair project.

Background Information

The black walnut belongs to the walnut family (Juglandaceae) (1), and there are currently 24.6 million of these trees scattered across Ohio (2). Even so, Ohio is the second-best state in numbers of black walnut trees, just shy of Missouri (2). Every black walnut tree can drop from 60 pounds up to 350 pounds of nuts each fall depending on the species (1, 2). Each nut consists of a tough outer hull that can be green or yellow in color and turn black when fully ripened and dried. The nuts inside are edible and are primarily used in natural dishes to give out a nutty flavor. However, the walnuts contain 5-hydroxy-1,4-naphthoquinone or simply called ‘*juglone*’ (**Fig. 1**), which turns out to be the reason that plants don't grow near the tall, dark tree (3-5). This compound is found in all parts of the tree, including the leaves, walnuts, and roots. Even if you were to take the tree out, the juglone that was in the tree is still present in the soil for a few extra months. These hulls and nuts don’t have a big commercial purpose, thus making them wasted every year. There are few reports suggesting that these walnut hulls can be used as a fresh mulch, but nothing significant.

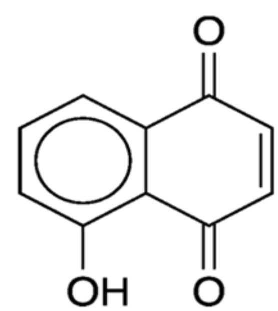


Fig. 1. The Chemical Structure of *Juglone* (Source: American Chemical Society)

In-Depth Review of Literature

A thorough search of literature showed what kind of plants are inhibited by juglone, and what other plants didn’t have any effect. For instance, tomato, potato, and pepper plants were potentially inhibited and raspberries, cherries, and blackberry plants were uninhibited (1-5). It is fascinating to know that this compound prevents the growth of certain plant species but facilitates others. However, there is not much information on plants like soybeans (*glycine*) and corn (*zea*) which are grown in abundance in Ohio. There is currently 5 million acres of *glycine* and 3.5 million acres of *corn* grown in Ohio every year (6, 7). For this project, I planned to test whether black walnut products can be utilized as a biofertilizer that is able to allow the growth of these commercial plants important for Ohio. I decided to test the effect of black walnut products on the germination and cellular respiration rates of *glycine* and *zea* seeds and growth of the respective established plants.

Research Question, Hypothesis, Goals, and Expected Outcomes

The objective of this project is to determine how the effect of juglone found in black walnuts affects the germination, cellular respiration rates, and growth of *glycine* and *zea*. **The hypothesis is that the extracts derived from walnut hull and/or nut powder will enhance the germination and cellular respiration rates of *glycine* and *zea*, and therefore the overall growth of the plants.** The goal of this project is to identify specific byproducts of black walnuts that can be utilized to enhance the growth of these commercial plants. Our expected outcome is that either the black walnut hull and/or nut extract will exhibit beneficial effects for at least some of the varieties of *glycine* and *zea*.

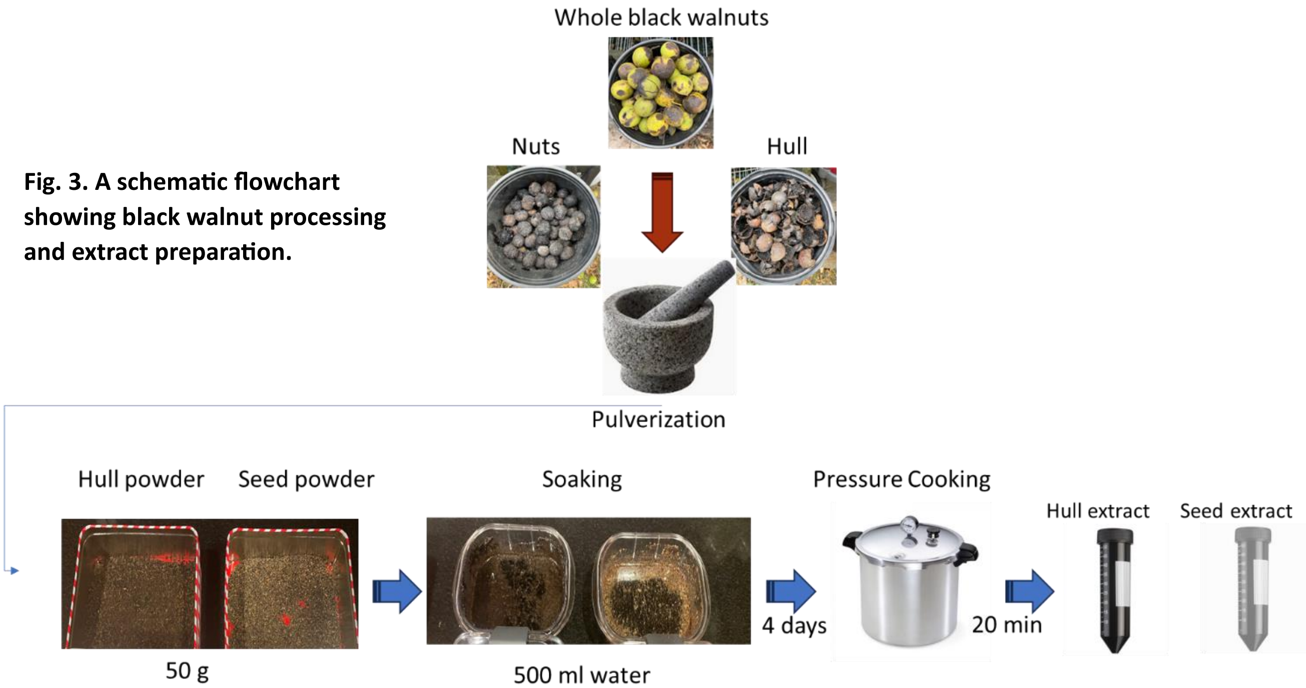
Materials and Methods

Preparation of black walnut extracts

Once I collected the whole black walnuts that I needed, I stored them in my sunroom for them to air dry because of the abundant number of squirrels that thrive in my backyard scavenging for all the black walnuts they can find, also preventing the rehydration of the walnuts from the rainfall. Dried and blackened walnut hulls and seeds were separated from whole walnuts and collected in separate bins (**Fig. 2**). Also, care was taken that the products contained no impurities such as leaves, sticks, etc. To pulverize the products, the walnuts were powdered using a variety of crushers such as a mortar and pestle, hammers, and manual grinding; and sieved the walnut powder to remove any coarse particles. This produced fine powders of hull and seeds. Approximately, 200 grams of each of these products were prepared and stored. Before experimentation, aqueous extracts of these two products were generated by soaking 50 grams of each of these products in a 500 milliliters of tap water. The final extracts were prepared by filtration with cheesecloth, and the solutions are stored in fifty milliliter conical tubes at room temperature (**Fig. 3**).

Fig. 2. Collection of black walnuts (10/8/23) and separation of hulls from nuts.

Fig. 3. A schematic flowchart showing black walnut processing and extract preparation.



Germination of Seeds

The organic *glycine* seeds were purchased from Be Still farms through Amazon, and the Ambrosia, True Gold, and Painted Hill varieties of *zea* seeds were purchased from a Straders store in Ohio. Germination containers were purchased from Lowe's gardening section. Tap water was used for soaking the seeds for 24 hours to accelerate germination. Briefly, ten seeds per condition were soaked (in tap water (control), hull extract, or seed extract) in a filter paper placed in separate containers at a warm place until germination. The time from sowing to germination of seeds and the number of germinating seeds per container were recorded.

Extract Treatments to Plants

Freshly germinated seeds were grouped into different batches and labeled with each group containing at least three to five seedlings. The first group was used as a control with no treatments. Groups two to five in each of the experimental arms (*glycine* and three varieties of *zea*) were treated with equal volumes of hull or seed extracts prepared as described above. Care was taken to not let the roots dry by replenishing with equal amounts of tap water or black walnut extracts at regular intervals.

Measurement of Cellular Respiration

Cellular respiration was measured using Vernier CO_2 probes. The CO_2 generated was recorded with the Vernier Graphical Analysis 4 App to indirectly measure the oxygen utilized and energy produced by these plants during germination. Each group of seedlings were placed one by one in small boxes and dried for one minute before the Vernier Graphical Analysis probes were placed on top for a duration of three to six minutes. Once the measurements were recorded, the small boxes were then air dried for one minute and the process repeated for the next batch of seedlings.

Data Collection

The number of germinations in each of the containers were recorded. In addition, the rate of plant growth was measured in terms of shoot and root lengths from the time of germination to day 3 and day 4 using a ruler. The cellular respiration data output was graphed using the Vernier Graphical Analysis 4 App for various conditions over the course of a three to six-minute duration and graphs were imported to Microsoft PowerPoint slides.

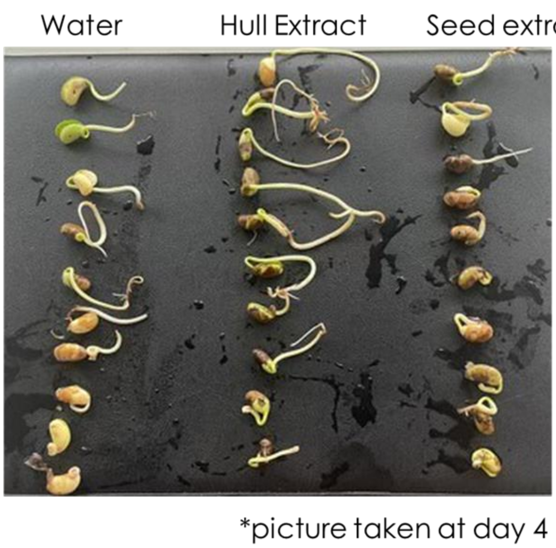
Data Analysis

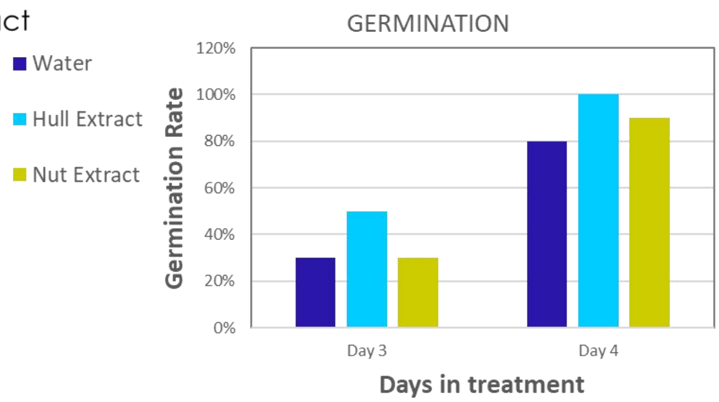
The efficiency of germination, changes to cellular respiration, and rate of growth of *glycine* and *zea* seedlings were analyzed using Microsoft Excel by comparing control with treated conditions.

Results

Effect of black walnut extracts on *Glycine max*

Effect on germination: Both hull and seed extracts stimulated the germination of *glycine* seeds with the hull extract showing an earlier effect (day 3 onward) than the seed extract (day 4 only). Germination efficiency was 80% in control, 100% in hull extract, and 90% in seed extract at day 4 (**Fig. 4**).





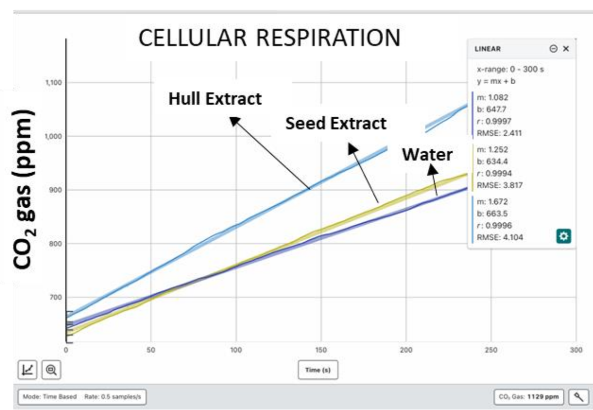
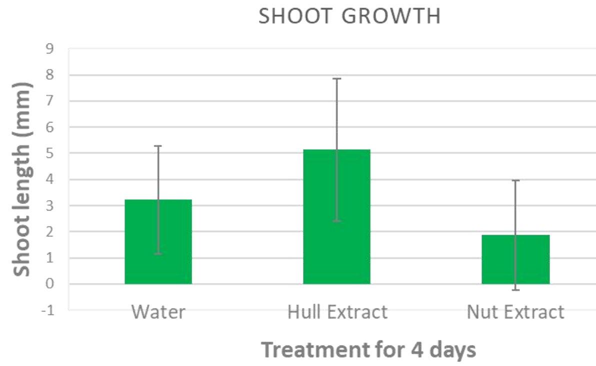


Fig. 4. Germination of *glycine* seeds (top left) under control and extract treated conditions. The germination efficiency of *glycine* seeds is plotted (top right). The CO_2 release levels from the three groups of *glycine* seedlings in time were plotted (bottom right). Images shown were taken with the Vernier Graphical Analysis app**

Effect on cellular respiration: Both hull and seed extract-treated *glycine* seedlings increased the amount of CO_2 (in ppm) release. At 200 seconds, the control released ~860 ppm of carbon dioxide, the hull extract released ~1000 ppm of CO_2, and the seed extract released ~900 ppm of CO_2 (**Fig. 4**).

Effect on growth: The hull extract increased both the shoot and root lengths of *glycine* plants. The seed extract increased the root length but decreased the shoot length compared to



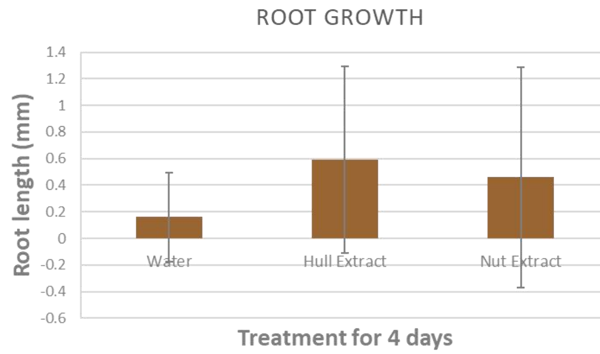


Fig. 5 *Glycine* plant growth (top left) under control and extract treated conditions. The shoot (top right) and root lengths (bottom right) of *glycine* plants under control and treated conditions are plotted.

the control. The hull extract roughly doubled the shoot length, but the seed extract roughly cut down the shoot length to one-half. Also, hull extract tripled the root length and seed extract doubled the root length of *glycine* (**Fig. 5**).

Effect of black walnut extracts on *Zea mays* varieties

Effect on germination: At day 4, 30% of Ambrosia and Painted Hill seeds germinated, and True gold didn't start germination. Hull extract increased the germination of Ambrosia and True Gold but not Painted Hill at day 4. Seed extract increased the germination of all three varieties at day 4. Between Ambrosia and True Gold, the hull extract showed a higher germination rate for Ambrosia (70%) than True Gold (40%) when compared to their respective controls. Among the varieties, the seed extract showed higher germination rates for Ambrosia (90%) and True Gold (70%) but had a negative impact on Painted Hill (0%) when compared to their respective controls (**Fig. 6**).

Effect on cellular respiration: The control CO_2 levels for Ambrosia, True Gold, and Painted Hill at 200 seconds were ~1020 ppm, ~1010 ppm, and ~1220 ppm, respectively. The hull extract levels for Ambrosia, True Gold, and Painted Hill at 200 seconds were ~1300 ppm, and ~1290 ppm, respectively. The seed extract for Ambrosia, True Gold, and Painted Hill at 200 seconds were ~1290 ppm, ~1500 ppm, and ~1300 ppm respectively. Thus, both hull and seed extracts showed an increased release of CO_2 (**Fig. 6**).

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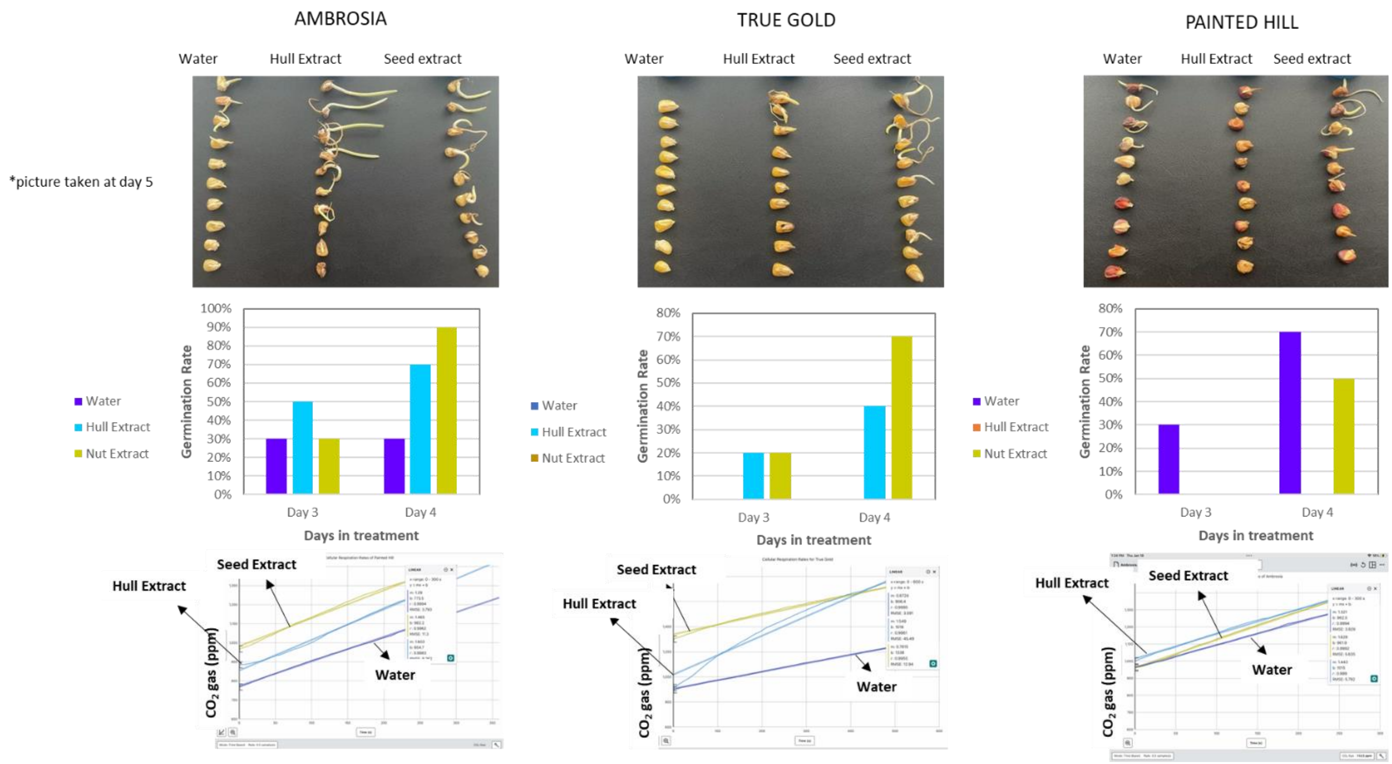

Fig. 6. Germination and cellular respiration rates of *zea* seed varieties. The images of germinating seeds after water or extract treatment (top row). The germination efficiencies of water and extract-treated *zea* varieties are plotted (middle row). The CO_2 release levels from the three groups of *zea* seedlings (in each of the three varieties) in time were plotted (bottom row). Images shown were taken with the Vernier Graphical Analysis App**

Effect on growth: The control shoot measurements were 9 mm, 1 mm, and 6 mm for Ambrosia, True Gold, and Painted Hill, respectively. The controls for the root measurements were 4 mm, 1 mm, and 5 mm, respectively. The hull extract for the shoot lengths were 30 mm, 6 mm, and 1 mm, respectively. The hull extract for the root lengths were 15 mm, 4 mm, and 1 mm, respectively. Thus, the hull extract significantly increased the shoot and root lengths of Ambrosia and True Gold but decreased the shoot and root length of Painted Hill. The seed extract for the shoot lengths were 8 mm, 5 mm, and 7 mm, respectively. The seed extract for the root lengths were 3 mm, 12 mm, and 9 mm, respectively. Thus, the seed extract increased the shoot and root lengths of True Gold and Painted Hill but not Ambrosia (**Fig. 7 & 8**).

*picture taken at day 10

Fig. 7. Effect of black walnut extracts on Ambrosia plant growth. Note the hull extract facilitated growth of Ambrosia but not the seed extract. Also note the seeds were stained (*brown*) in extract treated conditions.

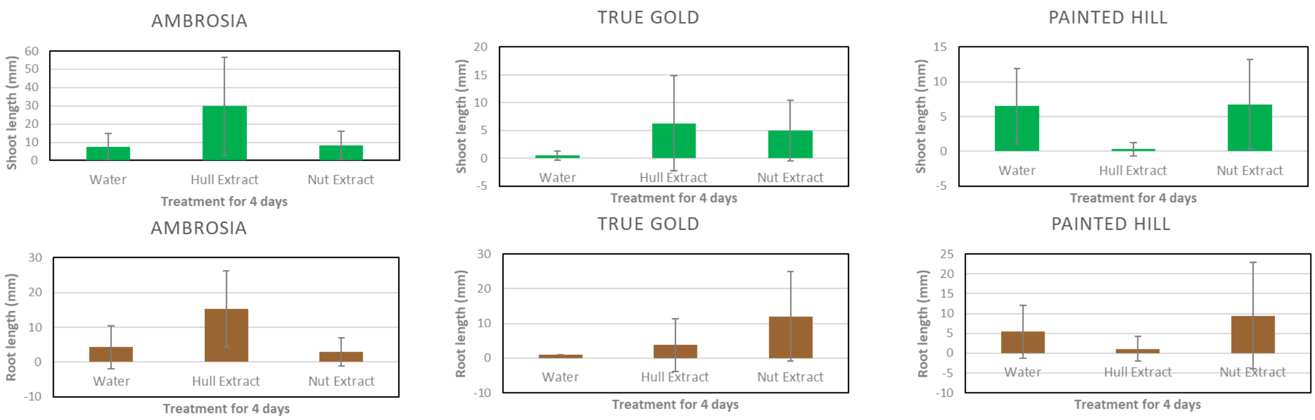

Fig. 8. Effect of black walnut extracts on the shoot and root lengths of *zea* varieties. Note the hull extract increased the shoot and root lengths in Ambrosia and True Gold but not in Painted Hill. Contrarily, the seed extract showed an increase in the root lengths of True Gold and Painted Hill.

Discussion & Conclusions

For both *glycine* and *zea* varieties, the hull extract showed an overall improvement in the germination efficiency, CO_2 release, and shoot length of plants while the seed extract stimulated only the root length but no other parameters (germination efficiency, shoot length, CO_2 release). After additional literature search (8-10), I found out that there is more juglone in the seeds (nuts) than the hull of black walnuts. Relating this to my data, this is not surprising because since the hull extract had a smaller amount of juglone present, the *glycine* and *zea* seeds treated with the hull extract were performing much better than those treated with the seed extract. Alternatively, the hull extract may contain more nitrogen and essential nutrients than the seed extract, which could stimulate the germination and growth of the *glycine* and *zea* varieties. Although the seed extract did not stimulate the germination abilities of *glycine* and most *zea* varieties (except for Painted Hill) or even the shoot lengths of these plants, surprisingly, it did stimulate root growth in many of these plants. It is likely that the seed extract may contain more phosphorus and potassium levels than the hull extract, which are the main nutrients that stimulate root growth. Nevertheless, our results suggest that whole black walnut extracts can be utilized, either separately (hull or seed extract) or together (hull and seed extract) for stimulating germination and promoting the growth (shoot and root systems) of *glycine* and *zea* varieties. Care must be taken however for the use of hull extract for certain varieties of *zea* plants such as the Painted Hill where it inhibited growth whereas the seed extract faired better in stimulating germination and growth. We anticipate that future studies in this area would aid in the further development of a clearer idea on what strains of *glycine* and *zea* would maximally benefit from such treatments and whether further processing of black walnut products (e.g., to inactivate juglone) may additionally promote *glycine* and *zea* growth. In summary, the current study sheds new light on the potential of using black walnut products as biofertilizers in Ohio *glycine* and *zea* fields that may be of commercial benefit to farmers while allowing agricultural waste management.

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